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PCT/AU99/00062

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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PP 5046 for a patent by MONASH UNIVERSITY and POLYCHIP PHARMACEUTICALS PTY LTD filed on 4 August 1998.

PRIORITY DOCUMENT

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WITNESS my hand this Twenty-sixth day of February 1999

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MANAGER EXAMINATION SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s): MONASH UNIVERSITY
and
POLYCHIP PHARMACEUTICALS PTY LTD
A.C.N. 006 455 456

Invention Title: THERAPEUTIC COMPOUNDS

The invention is described in the following statement:

THERAPEUTIC COMPOUNDS

This invention relates to novel structural analogues and derivatives of compounds with general analgesic or related pharmacological activity. In particular the invention relates to derivatives of opioid compounds, particularly morphine and related compounds.

BACKGROUND OF THE INVENTION

A large range of therapeutic compounds is currently used in the treatment of conditions such as allergies, diarrhoea, migraine and other pain conditions, and in the treatment of congestive heart failure. These compounds include compounds with analgesic or related activities, such as anti-tussives, anti-depressants, local anaesthetics, anti-hypertensives, anti-asthmatics, anti-histamines, and anti-serotonins.

However, many of the therapeutic compounds of the types enumerated above have undesirable side-effects, such as the respiratory depression caused by opiates. In particular, many drugs which are useful for their action on the peripheral nervous system have undesirable effects in the central nervous system.

Thus opiates are the most powerful analgesics known, but their usefulness is greatly limited by their side-effects, including severe respiratory depression, and ability to induce addiction and physical dependence.

Despite intensive efforts to design analogues of morphine and related opioids which retain the analgesic activity but which do not have a deleterious effect on the central nervous system and the bowel, success has been limited. Structure-activity relationships have been extensively investigated, and a number of features have been widely accepted as essential. See for example "An Introduction to Pharmacology" by J.J. Lewis (E. & S. Livingston Ltd, 1964 Pages 401-407), and "Principles of Drug Action: The Basis of Pharmacology (Ed. W.B. Pratt and

P.Taylor; Churchill Livingstone, 3rd edition, 1990, Pages 25-27). In particular, it is generally considered that to retain analgesic activity the group on the tertiary nitrogen should be small, and should preferably be methyl; larger substituents are likely to be opiate receptor antagonists rather than agonists. Thus replacement of the methyl group of morphine by an allyl or cyclopropylmethyl moiety produces an antagonist. Although there are some exceptions to this rule, such as N-amylnormorphine and N-hexylnormorphine, in general a large substituent will result in antagonist activity.

We have attempted to modify the ability of biologically-active compounds to cross the blood-brain barrier by incorporating a highly polar group into the molecular structure. Thus we have shown that derivatives of the 2N atom of mianserin comprising a guanidino group show H_1 and 5-hydroxytryptamine activity, but show no detectable activity in the central nervous system. In contrast, a compound in which the 2N atom of mianserin was substituted with a urea group still showed pronounced central nervous system activity (Jackson *et al*; Clin. Ex. Pharmacol. Physiol., 1992 19 17-23 and our U.S. Patent No. 5,049,637).

Naltrexamine and oximorphamine have been modified by incorporation of groups which are zwitterionic at biological pH in order to restrict access to the central nervous system (Botros *et al*; J. Med. Chem., 1989 32 2068-2071, and Portoghese, U.S. Patent No. 4,730,048). In US-4,730,048 the zwitterionic group was added at C6. Some of these analogues were full agonists, and one was a strong antagonist.

A bis(t-butyldimethylsiloxy)-substituted compound in which a guanidino derivative was attached to the nitrogen via a 3 carbon spacer chain was found to show no opioid activity at μ-receptors in isolated guinea-pig ileum (Jackson *et al*, 1992). This suggested that such compounds would not have the desired activity.

Therefore there is a need for therapeutic compounds which have less activity within the central nervous system, thus having fewer undesirable side-effects, whilst at the same time having greater specificity of action on peripheral physiological mechanism. We have found that several compounds with the general formula outlined below not only have reduced central side-effects, but retain activity at desired peripheral receptors. In particular, those compounds which show activities at opioid receptors retain broad analgesic activity, contrary to current orthodoxy which teaches that the analgesic effects of opioids are mediated from the CNS. Their selectivity for peripheral opioid receptors not only makes them useful for the treatment of pain without sedative or addictive effects, but also may make them useful for treatment of AIDS and related immune deficiency diseases.

SUMMARY OF THE INVENTION

In its broadest aspect, the invention provides an opioid compound of general formula I

[opioid-N]-[spacer]-[charged group],

I

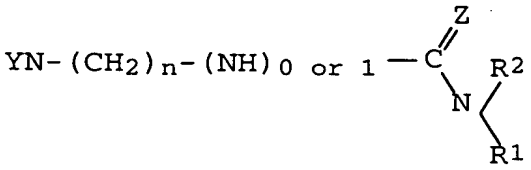
in which an opioid compound is linked via the nitrogen at position 17 to a spacer group, which in turn is linked to a charged group.

For the purposes of this specification, the term "opioid compound" is to be taken to mean a compound structurally related to morphine. The opioid compound preferably, but not necessarily, has opioid agonist or antagonist activity at opioid receptors.

The spacer can be any spacer group of dimensions approximately equivalent to an alkyl chain of 1 to 6 carbon atoms, and may for example be a straight or branched alkyl, alkenyl or alkenyl chain of 1 to 6 carbon atoms, which may

optionally be substituted. The spacer may also comprise a cyclic alkyl, alkenyl or alkynyl group. Preferably the spacer group is unsubstituted, and more preferably is of 2 to 3 carbon atoms. The charged group may be any group which has the ability to restrict access of the compound of formula I to the central nervous system, and is preferably an amidine or guanidine group.

According to one embodiment, the present invention provides an opioid compound of general formula (II)



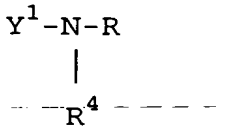
in which

YN- represents an organic residue obtained by removal of the R group from an opioid compound of general formula

(IIIa)

wherein R is H, alkyl of 1 to 6 carbon atoms, or cyclopropylmethyl,

or of the general formula



(IIIb)

wherein R^4 is methyl or ethyl, and

Y^1-NR^4 represents the corresponding organic residue;

Z is O, S or NR^3;

R^1 is H_1, alkyl or aryloxyalkyl, wherein the aryl group is optionally substituted by alkyl, alkoxy, halogen,

or alkyl substituted by halogen, and alkyl, alkoxy and the alkyl moiety of aryloxy alkyl have 1 to 6 carbon atoms;

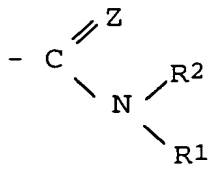
R^2 is H or alkyl with 1 to 6 carbon atoms;

R^3 is H, alkyl, hydroxy, amino, cyano or acyl, wherein alkyl and acyl have 1 to 6 carbon atoms;

n is an integer of 1 to 6,

and wherein

R^1 and R^3 may together complete an addition ring; then the grouping



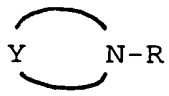
may become a heterocyclic moiety such as 2-imidazolyl or 2-imidazolinyl:

Preferably R is CH_3.

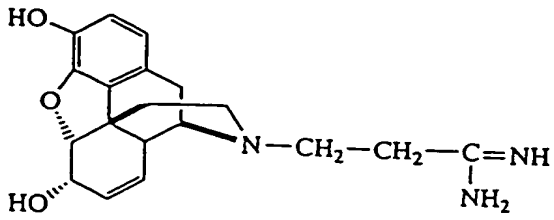
Preferably n is 2 or 3.

Preferably Z is NH, and R^1 and R^2 are both H.

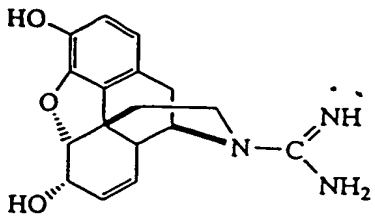
In order to indicate the trivalent N-atom more clearly, the structure of compounds of the formula (IIIa) may be written

 (IIIc)

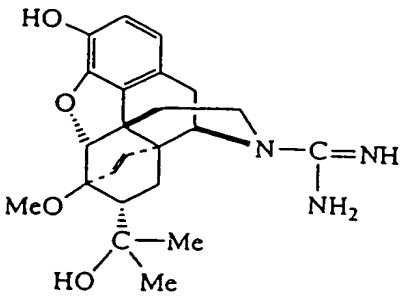
In a preferred embodiment, the compound of general formula I is one of the following:



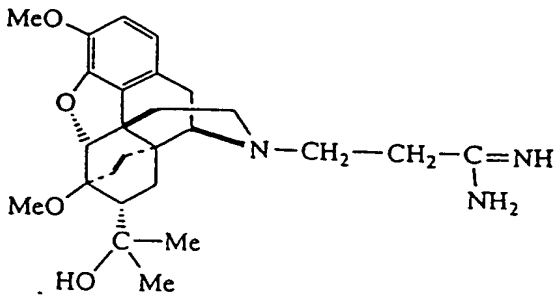
KRS-41



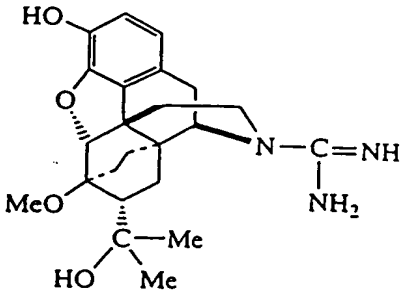
KRS-2-19



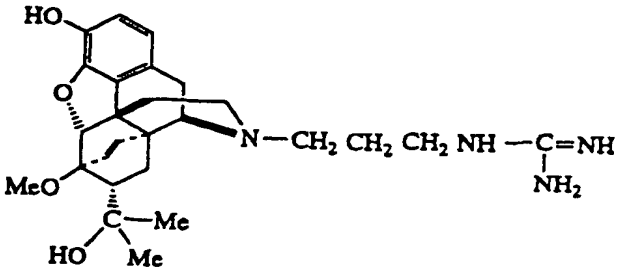
KRS-3-23-4



KRS-3-28



KRS-3-30-2



KRS-3-56

The precursors of YN- and Y^1NR^4- respectively are selected from compounds which are structurally related to morphine.

Thus the precursor of YN- or Y^1NR^4- is preferably a compound selected from the group consisting of morphine, codeine, heroin, ethylmorphine, O-carboxymethylmorphine, O-acetylmorphine, hydrocodone, hydromorphone, oxymorphone, oxycodone, dihydrocodeine, thebaine, metopon, etorphine, acetorphine, ketobemidone, ethoheptazine, diprenorphine

(M5050), buprenorphine, phenomorphan, levorphanol, pentazocine, eptazocine and metazocine.

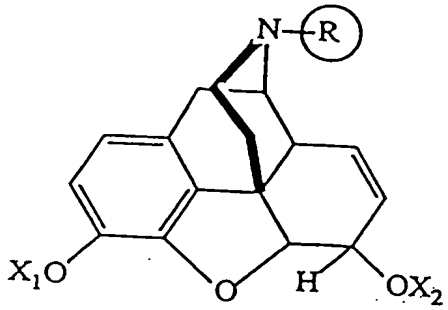
Preferably the precursor is morphine, codeine or buprenorphine.

Typical examples of morphine-related compounds of the formula (IIIa) or (IIIc) are illustrated in Table 1. In each case the group R has been circled in order to clearly identify the residue YN- or Y^1NR^4- as the remainder of the molecule.

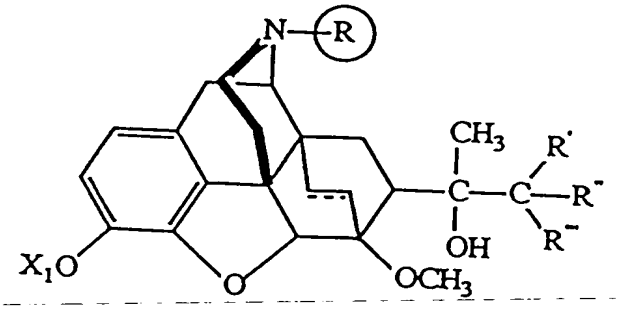
The preferred precursors also include the unnamed compounds whose structures are shown in Table 1.

Table 1

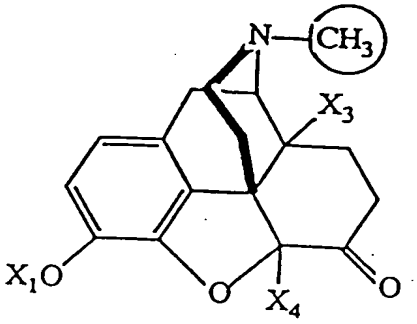
Compounds with Analgesic or Related Type Activity and Some Related Structures



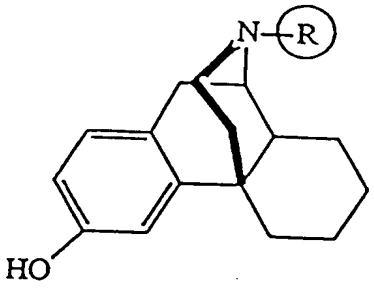
R	X_1	X_2	Name
CH_3	H	H	Morphine
"	CH_3	H	Codeine
"	Et	H	Ethylmorphine
"	Ac	Ac	Heroin
"	CH_2COOH	H	O-Carboxymethylmorphine
"	Ac	H	O-Acetylmorphine
"	$tBuMe_2Si$	$tBuMe_2Si$	"Disilyl" morphine
H	$tBuMe_2Si$	$tBuMe_2Si$	"Disilyl" normorphine



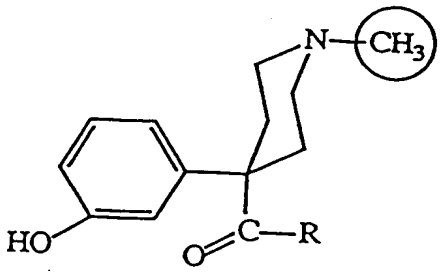
R	X	- or =	R'	R''	R'''	Name
CH_3	H	=	H	H	Et	Etorphine
"	Ac	=	H	H	Et	Acetorphine
"	H	-	H	H	Et	-
"	Ac	-	H	H	Et	-
CH_2-◁	H	=	H	H	H	Diprenorphine
"	H	=	CH_3	CH_3	CH_3	Buprenorphine



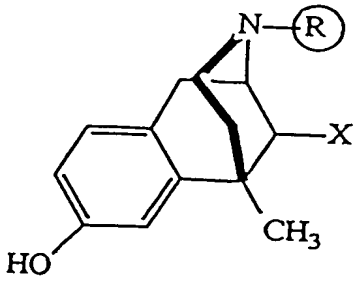
X_1	X_3	X_4	Name
CH_3	H	H	Hydrocodone
H	H	H	Hydromorphone
H	OH	H	Oxymorphone
CH_3	OH	H	Oxycodone
H	H	CH_3	Metopon



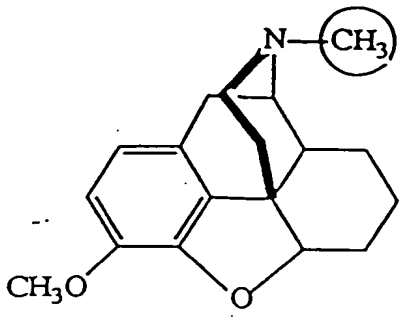
R	Name
$PhCH_2CH_2$	Phenomorphan
CH_3	Levorphanol



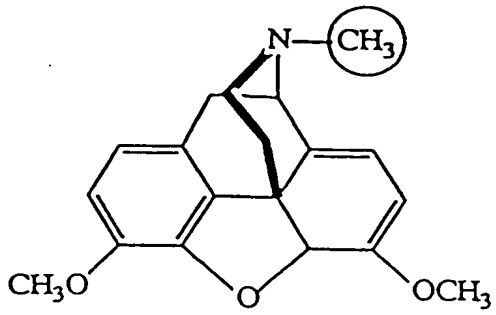
R	Name
CH_3CH_2	Ketobemidone
CH_3CH_2O	Ethoheptazine



R	X	Name
CH_3	CH_3	Ketobemiodone
H	CH_3	Eptazocine
$Me_2C=CHCH_2-$	CH_3	Pentazocine



Dihydrocodeine



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Thus the invention provides in a second broad aspect an opiate receptor agonist having analgesic properties and having reduced or no CNS activity. Preferably the opiate receptor agonist is a compound of general formula I or general formula II as defined above.

Where appropriate, the invention also includes pharmaceutically acceptable salts of the compounds of formula I, or formula II. A variety of pharmaceutically-acceptable salt-forming organic and inorganic acids is well known in the art.

According to a third aspect of the invention, methods for the preparation of the compounds of formula II are provided, as set out below, in which it will be appreciated that YN- may be replaced by Y^1NR^4-:

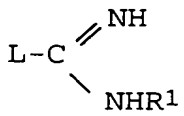
1. By the reaction of a compound of formula

YN-H (IV)

with a cyanamide, R^1NHCN, according to the equation

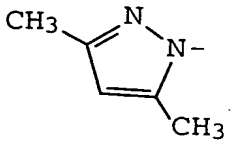
$$YN\text{-}H + R^1NHCN \rightarrow YN\text{-}C(=NH)\text{-}NHR^1$$

2. By the reaction of a compound of formula (IV) with a compound of formula



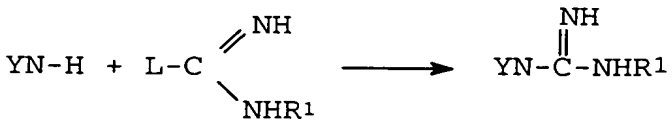
(V)

wherein L is a suitable leaving group, for example CH_3O, CH_3S, CH_3SO_2, SO_3H or



(3,5-dimethylpyrazol-1-yl)

according to the equation

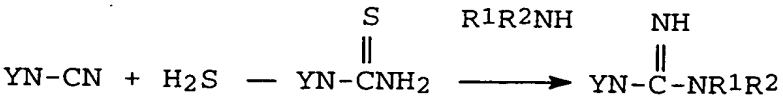


Compounds of the formula (II) wherein Z is S not only possess useful therapeutic activity *per se*, but may also be used as intermediates for the preparation of compounds of formula II wherein Z is NR^2, *eg.*

3. By the reaction of a compound of the formula

YN-CN (VI)

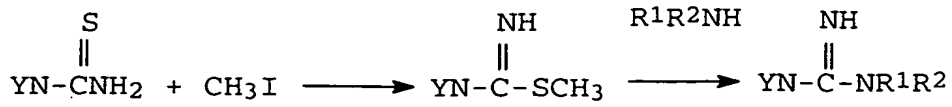
with H_2S there is obtained an N-thiocarboxamide $YN\text{-}CSNH_2$, which may be reacted with an amine R^1R^2NH according to the two-stage equation



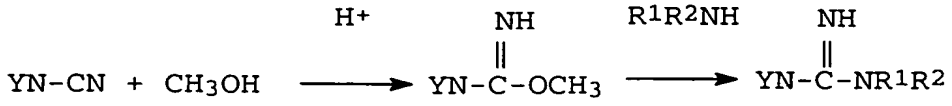
to yield compounds of the invention where Z is S and where Z is NH.

4. The N-thiocarboxamide may also be methylated, for example using CH_3I, to yield an isothiourea

compound, which in turn may be reacted with an amine R^1R^2NH to yield a compound of the invention:



5. An alternative method of synthesis of compounds of formula (II) comprises reacting an N-cyano compound of formula (VI) with methanol under acidic conditions to yield an isourea, which in turn is reacted with an amine according to the equation



6. Compounds according to formula (II) where Z is N may also be prepared, for example from the N-cyano compound of formula (VI) and the appropriate methyllated residue (for example, sodamide or methyllated amines):

7. Compounds of the formula (VI), most of which are also novel, and which are useful as intermediates in reactions 3, 5 and 6 above, are prepared by reacting a compound of formula (III) (see Table 1) with cyanogen bromide in a hydrocarbon solvent:

$$YN\text{-}R + BrCN \rightarrow YN\text{-}CN$$

8. Compounds of general formula (IV), which are useful as intermediates in reactions 1 and 2, are prepared from the compounds of formula (III) (Table 1) by the following reactions:

$$YN\text{-}R + Cl_3CCH_2OCOCl \rightarrow YN\text{-}CO.OCH_2CCl_3$$

$$YN\text{-}COOCH_2CCl_3 \xrightarrow{Zn/AcOH} YN\text{-}H$$

Some compounds of the invention are optically active, and it will be clearly understood that both racemic mixtures and isolated stereoisomers are within the scope of the invention.

According to a fourth aspect, the invention provides a composition comprising as an effective agent a compound according to formula I, together with a pharmaceutically acceptable carrier.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

According to a fifth aspect, the invention provides a method of inducing analgesia, comprising the step of administering an effective amount of a compound of the invention to a mammal in need of such treatment. The mammal may be a human, or may be a domestic, companion or zoo mammal. Preferably the mammal is a human.

The dosage to be used will depend on the nature and severity of the condition to be treated, and will be at the discretion of the attending physician or veterinarian. The most suitable dosage for a specific condition can be determined using normal chemical trial procedures.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

Brief Description of the Figures

Figure 1 shows dose-response curves for morphine-like activity in guinea-pigs stimulated ileum preparations, using morphine as standard

a) Compounds KRS-3-28 and KRS-3-30-2;
b) Compounds KRS-41 and KRS-2-19.
c) compound KRS-3-56

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples, and to the Figures.

Example 1 Preparation of N-Cyano Compounds, YN-CN

A solution of YN-R (0.02 mole of the base) in anhydrous benzene (20 ml) was added slowly to a stirred solution of cyanogen bromide (2.3 g) in anhydrous benzene (20 ml) in an atmosphere of nitrogen. After 24 hours, the mixture was diluted with diethyl ether (50 ml) and shaken with water (50 ml). The separated aqueous layer was back extracted with a mixture of benzene and ether (equal volumes of each, total 50 ml) and the combined organic layers dried over anhydrous potassium carbonate and then evaporated under reduced pressure. The residual solid was recrystallized from ethanol to give the N-cyano derivative YN-CN as colourless needles.

Example 2 Preparation of Carboxamidines, $YN{=}C(-NH_2){=}NH$

A solution of sodamide in liquid ammonia was prepared in the usual way from methyllic sodium (0.35 g) in dried liquid ammonia (150 ml) in the presence of a trace of ferric nitrate. The reaction mixture was kept at about -70°C and moisture was rigorously excluded. The N-cyano derivative YN-CN (0.01 mol) was then added slowly, and the mixture stirred whilst dried hexamethylphosphorictriamide (HMPA) was added dropwise until the N-cyano compound began

to dissolve; about 1 ml of HMPA was required. A deep brown solution was formed. The stirring was continued for 30 minutes and the solution poured cautiously into a solution of ammonium chloride (4 g) in iced water (150 ml). The resulting suspension was kept for some 30 minutes at room temperature and the solid then filtered off and washed with a little water. The residue (a) was reserved. The combined filtrate and washings were concentrated *in vacuo* to about 25 ml, when a second crop of solid (b) separated. The two crops (a) and (b) were combined and recrystallized from isopropanol to give the amide hydrochloride

$YN\text{-}C(=NH)\text{-}NH_2.HCl$ as the colourless solid.

Example 3 Preparation of Thiocarboxamido Derivatives, $YN\text{-}CSNH_2$

Dry hydrogen sulphide was passed through a solution of the N-cyano compound YN-CN (500 mg) in a mixture of triethylamine (0.25 ml) and pyridine (25 ml) for 24 hours. The resulting solution was poured into water (150 ml) and the mixture stirred for 30 minutes at room temperature to afford colourless crystals which were filtered off, washed with fresh water and dried in *in vacuo*. Recrystallization from a mixture of diethyl ether and light petroleum gave colourless needles of the desired compound.

Example 4 Preparation of Carboxoamido Derivatives, $YN\text{-}CONH_2$

A slurry of the N-cyano compound YN-CN (0.02 moles) in aqueous hydrogen peroxide (100 Vol., 0.51 ml) and 20% aqueous sodium hydroxide (0.51 ml) was stirred for 30 minutes, during which time the reaction mixture became warm, then cooled to room temperature; some oxygen was evolved. Three portions of methanol (3 x 2 ml) were added to the reaction mixture, at 30 minute intervals

with stirring. The mixture was warmed to 60°C for 15 minutes, then poured into water (50 ml) to give a white precipitate which was filtered at the pump, washed with water (2 x 10 ml) and dried *in vacuo* to give the N-carboxamido derivative YN-$CONH_2$ as a colourless solid.

Example 5 3,6α-Bis[dimethyl(1,1-dimethylethyl)siloxy]-7,8-didehydro-4,5α-epoxymorphinan

Dry, alcohol-free dichloromethane (100 ml) was added to a flask containing normorphine (5.42 g, 20 mmol), *t*-butyldimethylsilyl chloride (6.62 g, 44 mmol), imidazole (6.12 g, 90 mmol), and 4-dimethylaminopyridine (120 mg, 1.0 mmol). After 20 hours of stirring at room temperature, the reaction mixture was diluted with ether (200 ml), washed with water (3 x 200 ml), dried (Na_2SO_4), and evaporated to give a grey-yellow solid (10.11 g). Recrystallization from ethanol gave very fine grey needles (5.20 g, 52%), m.p. 105.7-107.0°C. The mother liquors were recrystallized (ethanol, twice) to give a second crop (2.45 g, 25%), m.p. 105.0-106.7°C. A small portion of the first crop was recrystallized again to give m.p. 106.2-107.2°C.

Example 6 Preparation of O,O'-Bis-t-butyldimethylsilyl-morphine

Ref: Neuvo., J. Chim. 1980 4 (6) 369-375

Solid t-butylchlorodimethylsilane (3.8 g, 25 mmol) was added to a stirred solution of morphine (3.0 g, 10.5 mmol) and imidazole (3.6 g, 52.9 mmol) in dimethylformamide (DMF; 20 ml) under a nitrogen atmosphere. Stirring of the reaction mixture was continued at room temperature for 2 hours, then the mixture was heated to 90° for 4 hours. The mixture was poured into water (25 ml) then extracted into dichloromethane (3 x 25 ml), dried (K_2CO_3) and evaporated to give a yellow oil, which crystallised on addition of a small amount of methanol. Recrystallisation from methanol gave colourless needles m.p. 118-119°C (Lit 119-119.5°C) (5.02 g, 93%).

Example 7 Preparation of N-Cyano-O-O'-bis-t-butyldimethylsilylnormorphine

A solution of bis-silylmorphine (7.0 g, 1.36 mmol) in dry benzene (50 ml) was added dropwise to a stirred solution of cyanogen bromide (2.9 g, 27.4 mmol) in dry benzene under a nitrogen atmosphere. The stirred solution was refluxed for 4 hours, allowed to cool to room temperature, then evaporated. The solid residue was purified by rotary chromatography (SiO: 5% ethanol in chloroform), then crystallisation from methanol to give N-cyano-O-O'-bis-t-butyldimethylsilylnormorphine (6.3 g, 86%).

Example 8 Preparation of O,O'-bis-t-butyldimethylsilyl-N-thiocarboxamidonormorphine

Cyanamide (524 mg, 1.0 mmol) and triethylamine (101 mg, 1.0 mmol) were dissolved in dry pyridine (20 ml). Dry hydrogen sulphide gas was slowly bubbled through the stirred pyridine solution for 4 hours, then the mixture was poured into water (100 ml), extracted into dichloromethane (3 x 20 ml), washed with water (3 x 20 ml), dried with $MgSO_4$, and evaporated. Recrystallisation from methanol gave colourless needles of the required O,O'-bis-t-butyldimethylsilyl-N-thiocarboxamidonormorphine (490 mg, 88%).

Example 9 Preparation of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-methylmorphinan

Solid t-butyldimethylsilyl chloride was added to a stirred solution of morphine (1.0 g, 0.0035 mmol) and imidazole (1.2 g, 0.052 mol) in DMF (7 ml) under nitrogen. Stirring of the reaction mixture was continued for 2 h at room temperature, and then the mixture was heated at 90°C for 4 h. After 4 h the reaction mixture was poured into water (25 ml) and was extracted into methylene chloride.

The organic layer was dried with potassium carbonate and was evaporated under reduced pressure. The yellow solid formed was purified by recrystallization with methanol. (Yield = 1.13 g, 72%).

Example 10 Preparation of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-N-cyanomorphinan

A solution of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-methylmorphinan (0.4 g, 0.76 mmol) in dry benzene (5 mL) was added dropwise to a stirred solution of cyanogen bromide (0.17 g, 1.53 mol) in dry benzene (5 mL) under nitrogen. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the solid residue was purified by recrystallization with methanol. (Yield = 0.34 g, 85%).

Example 11 Preparation of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-aminoiminomethyl)-morphinan

Ref: Ravi S. Garigipati, Tetrahedron Letters, Vol 31, No 14, pp 1969-1972, 1990.

J. I. Levin, E. Turos and S.M. Weinrub, Synthetic Communications, 12, 989-993, 1982.

A solution of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-N-cyano-morphinan (100 mg. 0.19 mmol) in dry benzene (2 ml) was added to a solution of methylchloroaluminum amide (prepared according to the Weinrub procedure) in benzene at room temperature. This solution was heated at 80°C under nitrogen for 20 h. The reaction mixture was cooled, and the aluminium complex was decomposed by carefully pouring the solution into a slurry of silica gel (2.0 g) in chloroform. The mixture was stirred for 5 min and filtered. The filter cake was washed with methanol (50 mL). Evaporation of the filtrate gave a

white solid (0.106 g), which was used in the next step without further purification.

Example 12 Preparation of (5a,6a)-7,8-didehydro-4,5-epoxy-17-(N-aminoiminomethyl)-morphinan-3,6,-diol. (KRS-2-19)

Ref: R. Newton, D.Reynolds, M. Finch, D. Kelly, S. Roberts, Tetrahedron Letters, No 41, 3981-82, 1979.

A slurry of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-aminoiminomethyl-morphinan (106 mg, 0.19 mmol) in 10:1 mixture of acetonitrile and tetrahydrofuran was cooled in an ice bath, and 40% aqueous HF (0.2 mL) was added dropwise. After stirring overnight at room temperature the reaction mixture was concentrated under reduced pressure to give a light yellow solid, which was passed through a short silica gel column using methylene chloride/methanol in 8:2 ratio as the eluent to give KRS-2-19 as a white solid (0.64 g, 98%).

Example 13 Preparation of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxymorphinan

Normorphine, prepared according to Chemical Abstracts, Vol. 54, 162f, (100 mg, 0.36 mmol) was dissolved in dry DMF (0.5 mL) and imidazole (0.0628 g, 0.92 mmol) and dimethylaminopyridine (0.07 g) was added. t-Butyldimethylsilyl chloride was then added in small amounts at room temperature. After the addition was complete the reaction mixture was stirred at room temperature under nitrogen while being monitored by thin layer chromatography. After 10-15 min distilled water was added and the reaction mixture was extracted with methylene chloride. The methylene chloride layer was dried over potassium carbonate and evaporated under reduced pressure to give crude product, which was purified by column chromatography on silica gel using methylene chloride/methanol/ammonium

hydroxide in 9:1:0.1 ratio as the eluent. (Yield = 120 mg, 65%).

Example 14 Preparation of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-cyanoethyl) morphinan

Ref: J.A.Bell and C. Kenworthy, Synthesis, 650-652, 1971.

3,6-Bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxymorphinan (0.26 g, 0.52 mmol) was dissolved in absolute ethanol (3 mL) and acrylonitrile (0.07 ml, 1.0 mmol) was added dropwise at room temperature. The reaction mixture was stirred at room temperature overnight, and the solvent was evaporated under reduced pressure to give a white solid (0.26 g, 90% yield).

Example 15 Preparation of 3,6,bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-aminoiminom ethyl-ethyl)morphinan

A solution of 3,6-bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-cyanoethyl) morphinan (0.257 g, 0.46 mmol) in dry benzene (5 mL) was added to a solution of methylchloroaluminum amide in benzene at room temperature. The solution was heated at 80°C under nitrogen for 20 h. This was worked up as before to give a white solid (0.157 g), which was used for the next step without further purification.

Example 16 Preparation of (5a,6a)-7,8-didehydro-4,5-epoxy-17-N-aminoiminomethyl-ethyl)-morphinan-3,6-diol. (KRS-41)

The crude 3,6,bis(t-butyldimethylsiloxy)-7,8-didehydro-4,5-epoxy-17-(N-aminoiminomethyl-ethyl)morphinan was deprotected using 40% HF in 10:1 mixture of acetonitrile and tetrahydrofuran as described before. The product was triturated with ethylacetate and with methanol. The remaining white precipitate was recrystallized with

ethanol and water to give KRS-41 as a white powder (90 mg) in 94% yield.

Example 17 Preparation of N-aminoiminomethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronorthebaine (KRS-3-7)

N-Cyano-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronorthebaine was prepared according to the method of Bently and Hardy, J. Am. Ch. Soc., 1967 89 3281-3292. This compound was reacted with methylchloroaluminum amide in benzene as described before. The crude product was purified by column chromatography on silica gel using methylene chloride/methanol/ammonium chloride in 6:1:0.1 ratio as the eluent to give KRS-3-7 as a white solid (56 mg. 91% yield).

Example 18 Preparation of N-aminoiminomethyl-ethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronorthebaine (KRS-3-28)

7a-(1-Hydroxy-1-methylethyl)6,14-endo-ethenotetrahydronorthebaine, prepared according to the method of Bently and Hardy (1967) *op.cit.*, was converted to the corresponding N-cyanoethyl compound in 96% yield by reacting with acrylonitrile in absolute ethanol.

N-2-Cyanoethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronorthebaine was then reacted with methylchloroaluminum amide in benzene as described above. The crude product was purified by column chromatography on silica gel using methylene chloride/methanol/ammonium chloride in 9:1:0.1 ratio as the eluting solvent to give KRS-3-28 (125 mg, 45 % yield).

Example 19 N-Aminoiminomethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydro-nororipavine (KRS-3-23-4)

3-O-Acetyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydrooripavine, prepared according to the

method of Bently and Hardy, *op.cit.*, was reacted with cyanogen bromide in dry methylene chloride to give 3-O-acetyl-N-cyano-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine in 97% yield. This compound was then reacted with methylchloroaluminum amide in benzene as described above. The crude product was purified by column chromatography on silica gel using methylene chloride/methanol/ammonium chloride in 6:1:0.1 ratio as the eluting solvent to give KRS-3-23-4 as a white solid (102 g, 34% yield).

Example 20 N-Aminoiminomethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethanotetrahydro-oripavine (KRS-3-30-2)

7a-(1-Hydroxy-1-methylethyl)-6,14-endo-ethanotetrahydro-oripavine was prepared by the method of Lewis, Narcotic Antagonists, Advances in Biochemical Psychopharmacology, 1974 8 123-136, Raven Press, New York. The 3-O-acetyl ester was prepared by the addition of acetic anhydride to a solution of the phenol in aqueous sodium hydroxide, and was obtained as a white solid. The O-acetyl ester was then reacted with cyanogen bromide in dry chloroform to give N-cyano-nororipavine derivative in 70% yield, which was then reacted with methychloroaluminum amide in benzene. The crude product was purified by column chromatography on silica gel using methylene chloride/methanol/ammonium hydroxide in 9:1:0.1 ratio. KRS-3-30-2 was obtained as a white powder in 30% yield.

Example 21 N-(aminoiminomethyl-aminopropyl)-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine (KRS 3-56)

a) Preparation of N-cyanoethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine

7a-(1-Hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine was prepared according to the method of K.W. Bentley and D. G. Hardy, Journal of the

American Chemical Society, 1967, 89, 3281-3292. This compound was reacted with acrylonitrile in absolute ethanol as described. The crude product was purified by column chromatography on silica gel using methylene chloride / ethyl acetate / methanol in 4:4:1 ratio as the eluent.

b) Preparation of 3-(t-butyldimethylsiloxy)-N-cyanoethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydro-nororipavine

Solid t-butyldimethylsilyl chloride (0.035 g, 0.227 mmol) was added in small amounts to a stirred solution of N-cyanoethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine (80 mg, 0.189 mmol), imidazole (0.015 g, 0.227 mmol) and 4-dimethylaminopyridine (0.005 g) in anhydrous dimethylformamide (0.5 ml) under a nitrogen atmosphere. After stirring for 1h at room temperature distilled water (10 ml) was added to the reaction mixture and the mixture was extracted with methylene chloride. The organic layer was dried over potassium carbonate and evaporated under reduced pressure. The solid formed was purified by column chromatography on silica gel, using ethyl acetate/X4 in 1:1 ratio as the eluent. (Yield = 79 mg, 78%)

c) Preparation of 3-(t-butyldimethylsiloxy)-N-aminopropyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydro-nororipavine.

3-(t-butyldimethylsiloxy)-N-cyanoethyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydro-nororipavine (100 mg, 0.186 mmol) in dry ethyl ether (2 ml) was added dropwise to lithium aluminum hydride (0.008 g, 0.223 mmol) in dry ether (2 ml). After stirring for 3 h at room temperature wet ether followed by 10% NaOH (1 ml) was added to the reaction mixture. The solution was filtered and the white precipitate was washed with ether. The ether layer was evaporated under reduced pressure to give the amine as a white solid (99 mg, 98%).

d) Preparation of 3-(t-butyldimethylsiloxy)-(N-aminoimino-methyl-aminopropyl)-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine

Ref: Michael S. Bernatowicz, Youling Wu and Gary R. Matsueda, Journal of Organic Chemistry, 1992 57 2497-2502

To a mixture of 3-(t-butyldimethylsiloxy)-N-aminopropyl-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine (0.196 g, 0.37 mmol), diisopropylethylamine (0.065 ml, 0.37 mmol) and 1H-pyrazole-1-carboxamidine hydrochloride (0.055 g, 0.37 mmol) was added anhydrous dimethylformamide (2 ml), and the reaction mixture was stirred at room temperature under nitrogen for overnight. The reaction mixture was evaporated to dryness under reduced pressure, and the crude product was chromatographed on silica gel. (Yield = 0.191 g, 88%).

e) Preparation of N-(aminoiminomethyl-aminopropyl)-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydro-nororipavine (KRS 3-56)

40% HF (0.3 ml, 0.0065 mol) was added dropwise to 3-(t-butyldimethylsiloxy)-(N-aminoiminomethyl-aminopropyl)-7a-(1-hydroxy-1-methylethyl)-6,14-endo-ethenotetrahydronororipavine (0.191 g, 0.3 mmol) in 10:1 mixture of acetonitrile /tetrahydrofuran (10 ml), and the reaction mixture was stirred overnight at room temperature. The white precipitate formed was filtered and was washed with acetonitrile and then with methanol to give KRS 3-56 as a white solid (0.135 g, 96%).

Example 22 Analgesic Activity

We have found evidence that these compounds have analgesic activity by showing stereoselectivity for peripheral opioid receptors. Thus, low subcutaneous or intraperitoneal doses of N-methylnalorphninium iodide

(10-300 µg/kg) showed analgesic activity in the mouse test of Hendershot and Forsaith (J. Pharmacol. Exp. Ther., 1959 125 237-240) and in the rat inflamed paw test of Randall and Selitto (Archs. Int. Pharmacodyn. Ther., 1957 111 409-419), whereas N-allylmorphinium iodide given in doses of 10 mg/kg was found to be inactive in both tests. S-methyllisothiocarbamoyl norheroin iodide was also active in both tests after administration of doses of 1-3 mg/kg.

Compound KRS-41 (Example 16) was tested for analgesic activity in two mouse analgesia models. In the first test, the test substance was administered to groups of 5 ICR derived male mice weighing 22 ± 2 g one hour before subplantar injection of formalin (0.02 ml, 1% solution). Reduction of the induced hind paw licking time recorded during the following 20 to 30 minute period by 50% or more indicates analgesic activity. Table 2 below shows that KRS-41 has analgesic activity at 3 times the morphine concentration, which is consistent with the relative opiate receptor activities discussed below in Example 23.

Table 2

Treatment	**% Reduction in Hind Paw Licking time**
Vehicle (5% DMSO/saline)	0
Morphine HCl (10 mg/kg)	100
KRS-41 (10 mg/kg)	12
KRS-41 (30 mg/kg)	75

In the second test, the test substance was administered to groups of 3 ICR derived male mice weighing 22 ± 2 g 30 minutes before injection of PQ (2 mg/kg). Reduction in the number of writhes by 50% or more per group of animals observed during the 5 to 10 minute period after PQ administration, relative to a vehicle treated control group, indicates analgesic activity. Table 3 below shows

that KRS-41 has analgesic activity at 5 times the morphine concentration.

Table 3

Treatment	% Reduction in Writhes
Vehicle (5% DMSO/saline)	0
Morphine HCl (3 mg/kg)	87, 73 (two tests)
KRS-41 (3 mg/kg)	18
KRS-41 (15 mg/kg)	93

Example 23 Guinea Pig Stimulated Ileum Preparation

Five compounds, KRS-41 (Example 16), KRS-2-19 (Example 12, KRS-3-28 (Example 18), KRS-3-30-2 (Example 20) and KRS 3-56 (Example 21) were tested for opiate activity in a standard guinea-pig stimulated ileum assay, using morphine as a standard.

Male Monash strain guinea-pigs were killed and the ileum removed. Segments (approxm. 1.5-2.5 cm) were mounted on tissue holders with in-built stimulating electrodes, and set up in 5 ml isolated organ baths containing Krebs solution of the following composition (mM): NaCl 118.4; KCl 4.1; $MgSO_4.7H_2O$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 11.1; $CaCl_2.2H_2O$ 2.5. The Krebs solution was bubbled with carbogen (95% O_2, 5% CO_2), and the preparations maintained at 37°C under 1 gram resting tension. The tissues were stimulated transmurally using single pulses of 0.5 ms duration at 0.2 Hz and 40 V from a Grass SD9 stimulator, and allowed to equilibrate under these conditions before the addition of drugs.

Cumulative dose-response curves to morphine (using increments of a half log unit) were obtained before obtaining cumulative dose-response curves to the test compounds. The results are shown in Figure 1.

Surprisingly, KRS-41 showed excellent activity compared to morphine (Figure 1b). This compound has an

aminoiminoethyl substituent on the tertiary N atom, and was expected to have either no activity or antagonist activity. KRS 3-56 (Figure 10) also showed even more striking activity, with a potency of approximately 6 times that of morphone, and was a full agonist of the μ opiate receptor.

Although KRS-3-28 had low potency compared to morphine, its activity in this assay is comparable to that of codeine. Codeine is metabolized *in vivo* to morphine, so its effect after oral administration is comparable to that of morphine given by injection. KRS-3-28 is expected to metabolize in similar fashion after oral administration to give a buprenorphine-like compound.

In contrast, KRS-2-19 (Figure 16) and KRS-3-30-2 (Figure 1a) showed only partial morphine agonist activity. It therefore appears that a spacer group in which n is 2 results in stronger opiate activity than a spacer in which n is 1.

Example 24 Effect of KRS 3-56 and KRS-41 on the Central Nervous System

The effects of compounds KRS-3-36 and KRS-41 on the central nervous system were compared with that of morphine using a standard Irwin test (Irwin, S.; Psychopharmacologic (Berlin), 1968 13 222-257). The relevant results are shown in Tables 4 and 5.

Table 4

Test	Vehicle	Morphine 10 mg/kg
Tail elevation	2.5 ± 0.7	7.0 ± 0.7
Respiratory rate	5.6 ± 0.2	4.1 ± 0.3
Positional Passivity	4.7 ± 0.3	8.7 ± 0.4
Grip strength	5.1 ± 0.4	3.7 ± 0.3
Corneal reflex	4.5 ± 0.2	2.9 ± 0.2

Table 5

Test	Vehicle	KRS-41 30 mg/kg	KRS-3-56 3 mg/kg
Tail elevation	4.4 ± 0.2	2.0 ± 0.4	2.4 ± 0.4
Respiratory rate	5.1 ± 0.2	5.1 ± 0.2	5.4 ± 0.2
Positional Passivity	4.7 ± 0.2	4.7 ± 0.3	5.4 ± 0.5
Grip strength	5.0 ± 0.3	5.1 ± 0.4	5.1 ± 0.2
Corneal reflex	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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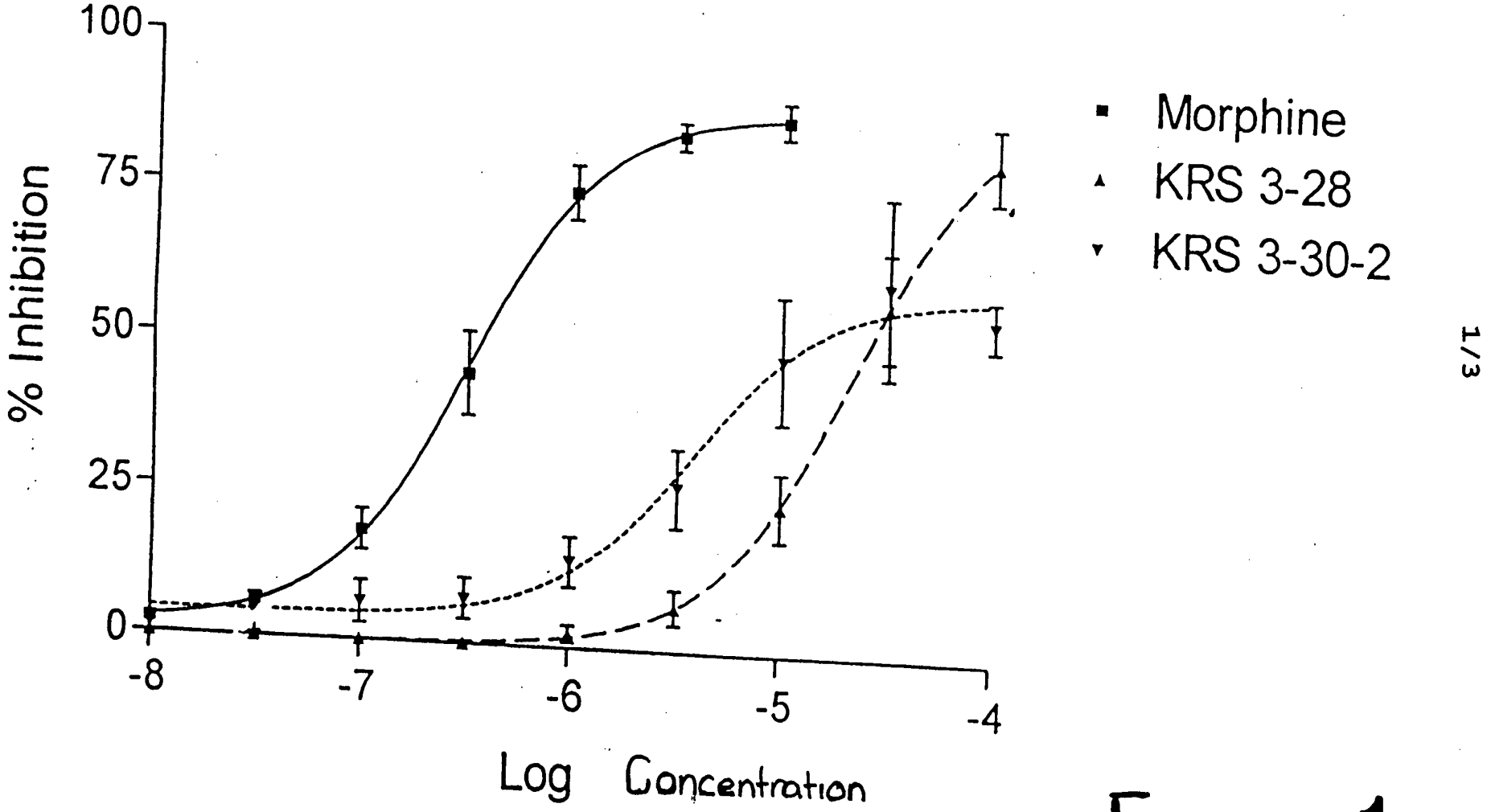
Dose response to morphine and KRS compounds in guinea-pig stimulated ileum, n=4.
100
75
50
25
0
% Inhibition
-8
-7
-6
-5
-4
Log Concentration
Morphine
KRS 3-28
KRS 3-30-2

FIGURE 1a

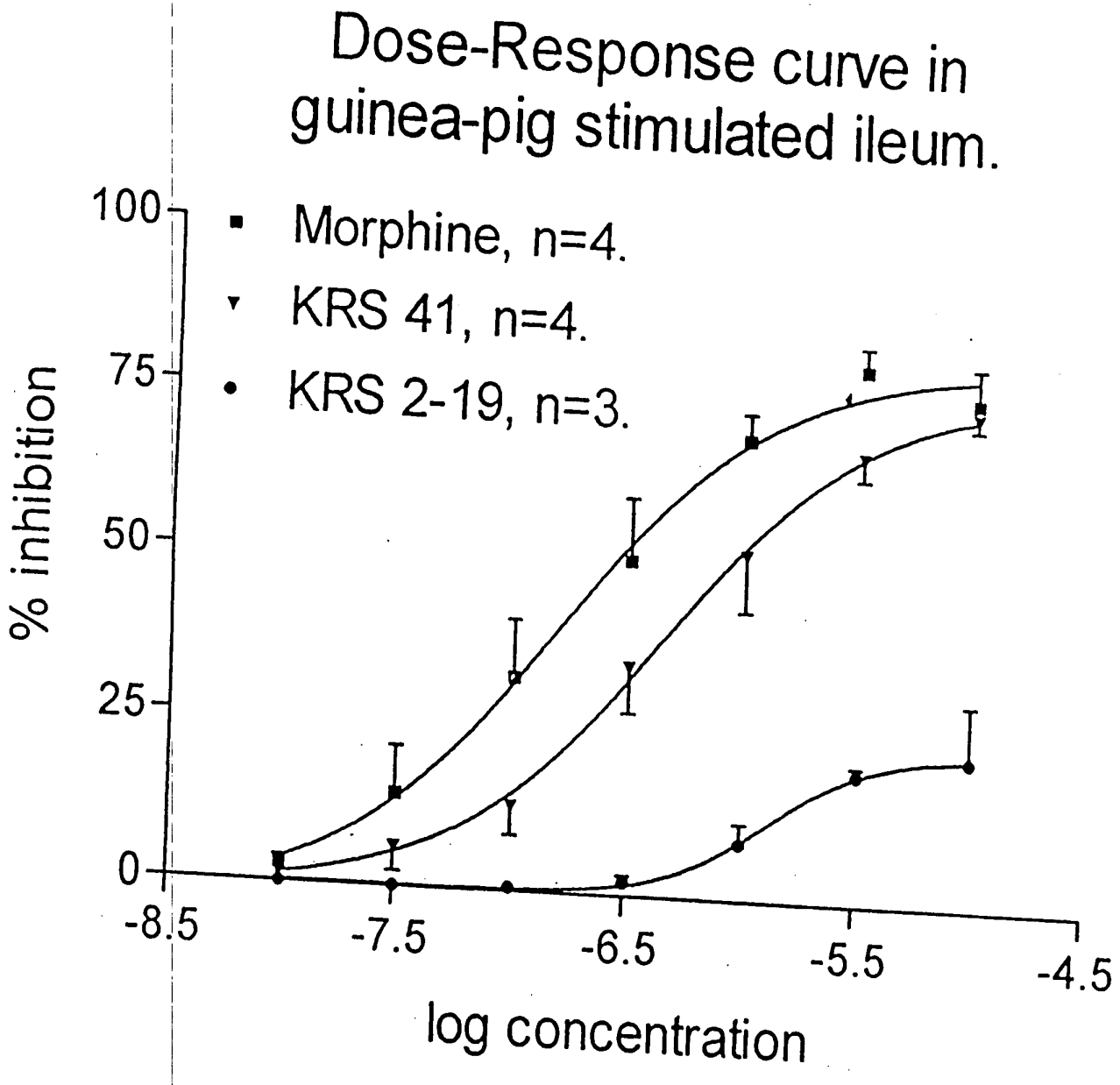
Dose-Response curve in guinea-pig stimulated ileum.
Morphine, n=4.
KRS 41, n=4.
KRS 2-19, n=3.
% inhibition
100
75
50
25
0
-8.5
-7.5
-6.5
-5.5
-4.5
log concentration

FIGURE 1b

Dose response curves to morphine and KRS 3-56 in guinea-pig stimulated ileum, n=3.

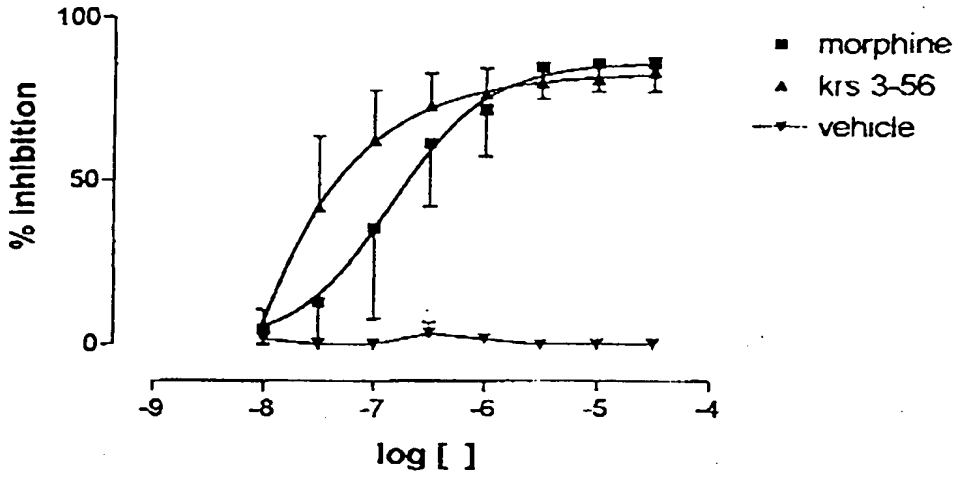

FIGURE 1C